American Journal of Hematology 51:240–242 (1996)

Haplotype Analysis of the Mexican Frameshift Cd 11 (−T) and −28 A→C β-Thalassemia Alleles

Francisco J. Perea, M. Amparo Esparza, Alma Rosa Villalobos-Arambula, Bertha Ibarra, and John M. Old

Divisiones de Medicina Molecular (F.J.P.) y Genética (A.R.V.-A., B.I.), Centro de Investigación Biomédica de Occidente, Hospital de Pediatría (M.A.E.), CMNO, IMSS, Guadalajara, México; Institute of Molecular Medicine, John Radcliffe Hospital, Headington, Oxford, England (J.M.O.)

The origins of the −28 A→C and frameshift Cd 11 −T (Fs Cd 11 −T) alleles were investigated by β-globin cluster haplotype analysis. These alleles were found in a Mexican mestizo family with β-thalassemia (β-thal). The −28 A→C mutation was described previously in Kurdish Jews linked to the most common haplotype in the world (+−−−−−++), the same haplotype observed in this Mexican family. Therefore, it is not possible to assess a new origin of the −28 A→C mutation in our population. The Fs Cd 11 −T allele, not reported to date in any other populations, was linked to the −++−−+− haplotype (sixth in frequency in the world). This haplotype has not been reported in association with any β-thal mutant, suggesting a Mexican origin for the Cd 11 −T mutation. © 1996 Wiley-Liss, Inc.

Key words: β-thalassemia, globin, haplotype

INTRODUCTION

β-thalassemia (β-thal) is one of the best characterized human genetic diseases at the molecular level. More than 120 different alleles are known [1], some with high frequency in certain populations—for instance, non-sense Cd 39 C→T and IVS-1 nt 110 (G→ A) in Mediterraneans [1]—while other alleles appear to be private to specific populations—for example, −28 A→C in Kurdish Jews and the 619 bp deletion in Asian Indians [1].

DNA haplotypes linked to the β-globin cluster (β-haplotypes) are characterized by five polymorphic sites 5′ to the β-globin gene (Hinc II 5′ to ε, Hind III at $^{G}\gamma$ and $^{A}\gamma$, Hinc II at Ψβ and 3′ to it) and two polymorphic sites to the β-globin gene (Ava II at β and Bam HI 3′ to β) [2]. The association of some alleles to specific β-haplotypes has been very useful in the elucidation of the origin and distribution of the β-thal alleles in the studied populations [2].

β-thal is considered uncommon in the Mexican population [3]; however, molecular studies showed ten different alleles, seven common in Mediterraneans and three observed in specific populations (Kurdish Jews, Asian Indians, and Mexicans) [4,5].

The β-haplotype analysis in a Mexican family with the −28 A→C and Fs Cd 11 −T alleles, previously identified in the proband [4], was performed to discover the origin of both mutations.

MATERIALS AND METHODS

The proband is a 16-year-old man, product of a second uncomplicated pregnancy and normal delivery. He presented anemia since early infancy and was diagnosed as the β-thal major at the age of 9 months. Both parents and one brother have the β-thal trait, whereas two other siblings are normal. The parents are unaware of any foreign ancestry, at least in three previous generations.

Blood counts and erythrocyte indices were determined by electronic cell counter. HbF was measured by alkali-denaturation and HbA_2 by microchromatography, and Hb electrophoresis was performed in cellulose acetate.

Molecular identification of the β-thal alleles was performed by the amplification refractory mutation system (−28 A→C) [6] and Rsa I digestion (Fs Cd 11 −T).

Received September 20, 1995; accepted October 11, 1995.

Address correspondence and reprint requests to B. Ibarra, División de Genética, CIBO, CMNO, IMSS, Apartado postal 1-3838, Guadalajara, Jalisco, Mexico.

TABLE I. Hematological and Biochemical Values of a Mexican Family With the Alleles −28 A→C y Frameshift Cd 11 (−T)

	HbF_B (%)	HbF_S (%)	HbA_2 (%)	RBC (10^{12}/l)	Hb (g/dl)	HTO (%)	MCV (fl)	MCH (pg)
I-1	1.41	1.2	5.5	7.03	13.0	45.0	64.0	18.4
I-2	2.27	2.38	5.0	5.68	11.7	39.7	69.9	20.5
II-1	4.40	4.38	5.6	5.50	9.5	32.9	59.9	17.2
II-2	1.20	1.32	2.5	1.82	5.4	15.4	84.7	29.7
II-3	1.10	1.12	2.2	4.86	13.0	41.3	84.9	26.7
II-4	0.97	1.46	2.4	5.39	13.2	42.9	79.6	24.7

HbF, fetal hemoglobin by Betke's and Singer's methods; HbA_2, α_2 δ_2; RBC, red blood cell count; HTO, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin.

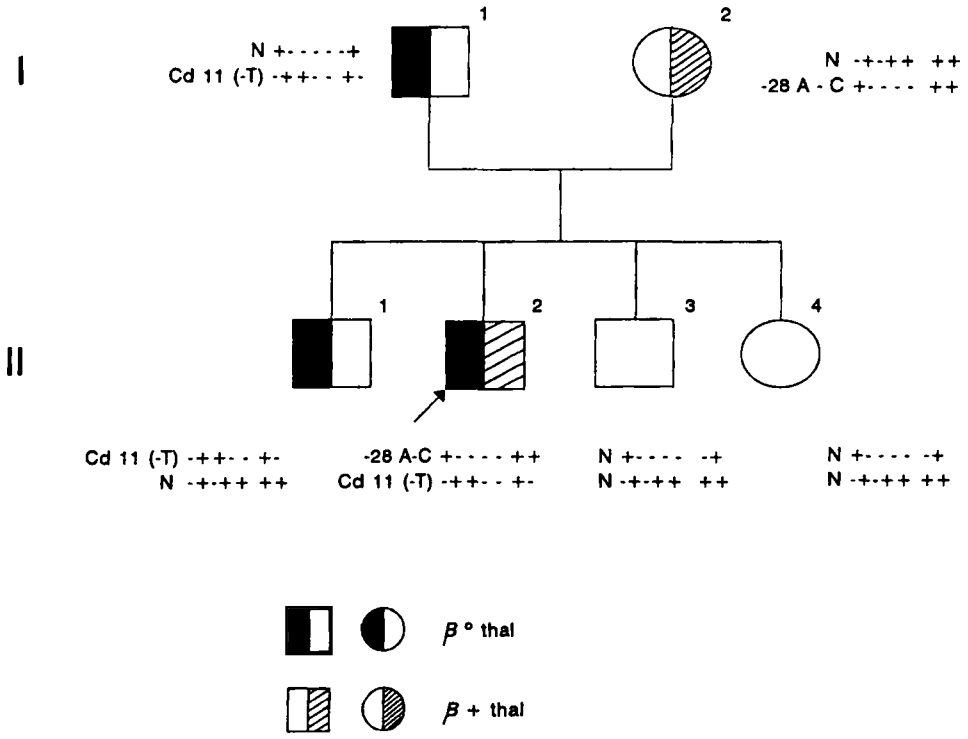

Fig. 1. Pedigree and β-haplotypes of the studied Mexican family with the −28 A→C, Cd 11 (−T) and normal alleles.

β-haplotypes were determined by analysis of seven polymorphic sites of the β-globin cluster amplified by polymerase chain reaction and digested with restriction enzymes: Hinc II 5′ to ϵ, Hind III at $^{G}\gamma$ and $^{A}\gamma$, Hinc II at Ψβ and 3′ to it, Ava II at β, and Bam HI 3′ to β [7].

RESULTS AND DISCUSSION

The results of the hematological and biochemical evaluations are shown in Table 1. The mother (I-2, Fig. 1) was a β^+-thal carrier with high HbA_2, moderate microcytosis, and normal HbF, with a −28 A→C heterozygote genotype. This mutant causes a two- to threefold decrease in transient expression in HeLa cells and a fourfold decrease in transcriptional in vitro assay [8].

The father and one brother (I-1 and II-1, Fig. 1) were β°-thal carriers with high HbA_2 and severe microcytosis (Table I); both were heterozygous for the Fs Cd 11 −T allele, which causes a frameshift with a termination codon seven codons downstream. The proband (II-2, Fig. 1) presented both abnormal alleles with a picture of β-thal major; to date he presents secondary hemosiderosis and cardiopathy.

The −28 A→C allele was shown to be associated with the +−−−−++ haplotype and the Cd 11 −T allele with the −++−−+− haplotype (Fig. 1). The +−−−−++

haplotype linked to the −28 A→C allele is the same described in Kurdish Jews; it was observed in 18/18 studied chromosomes [2], and ten of 12 alleles observed in Kurdish Jewish populations were associated with the same 5′ subhaplotype [2]. This subhaplotype, associated with the β^A gene [2], is the most common in all populations studied from around the world, including Mexicans (B. Ibarra, unpublished data). Even though the Kurdish Jews are a highly inbred population, it is not possible to assess a new origin of the −28 A→C allele in our population. The β-haplotype associated with the Fs Cd 11 −T has not been reported to date in association with any β-thal mutant [2]; it has been found in 1.1% of total world populations with the β^A gene (sixth in frequency). In Mexican mestizos it was observed with the β^A gene in 2% of the studied chromosomes, suggesting a Mexican origin.

REFERENCES

1. Thein SL: β-Thalassemia. In Higgs DR, Weatherall DJ (eds): Bailliere's Clin Haematol 6:151, 1993.
2. Flint J, Harding RM, Clegg JB, Boyce AJ: Why are some genetic diseases common? Distinguishing selection from other processes by molecular analysis of globin gene variants. Hum Genet 91:91, 1993.
3. Ibarra B: Talasemia en México. In: Enciclopedia Iberoamericana de Hematología, vol. 4. Salamanca: Ediciones Universidad de Salamanca, pp 645–648, 1992.
4. Economou EP, Antonarakis SE, Dowling CC, Ibarra B, de la Mora E, Kazazian HHJ: Molecular heterogeneity of β-thalassemia in mestizo Mexicans. Genomics 11:474, 1991.
5. Ibarra B, Perea FJ, Villalobos-Arambula AR: Alelos Talasemicos en pacientes mestizos Mexicanos. Rev Invest Clin 47:127, 1995.
6. Varawalla NY, Old JM, Sarkar R, Venkatesan R, Weatherall DJ: The spectrum of β-thalassaemia mutations on the Indian subcontinent: The basis for prenatal diagnosis. Br J Haematol 78:242, 1991.
7. Varawalla NY, Fitches AC, Old JM: Analysis of β-globin gene haplotypes in Asian Indians: Origins and spread of β-thalassemia on the Indian subcontinent. Hum Genet 90:443, 1992.
8. Surrey S, Delgrosso K, Malladi P, Schwartz E: Functional analysis of a β-globin gene containing a TATA box mutation from a Kurdish Jew with β-thalassemia. J Biol Chem 260:6507, 1985.